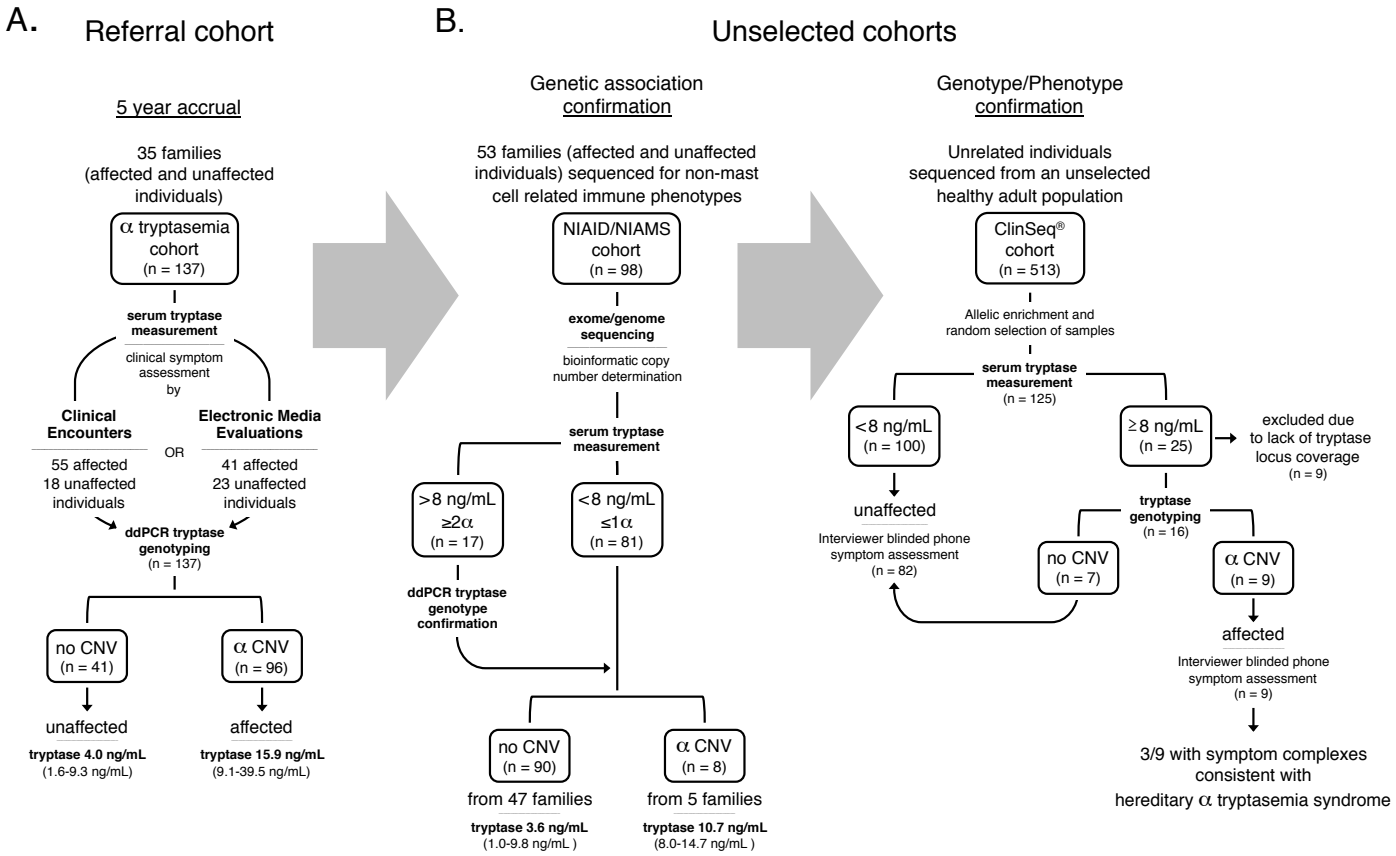
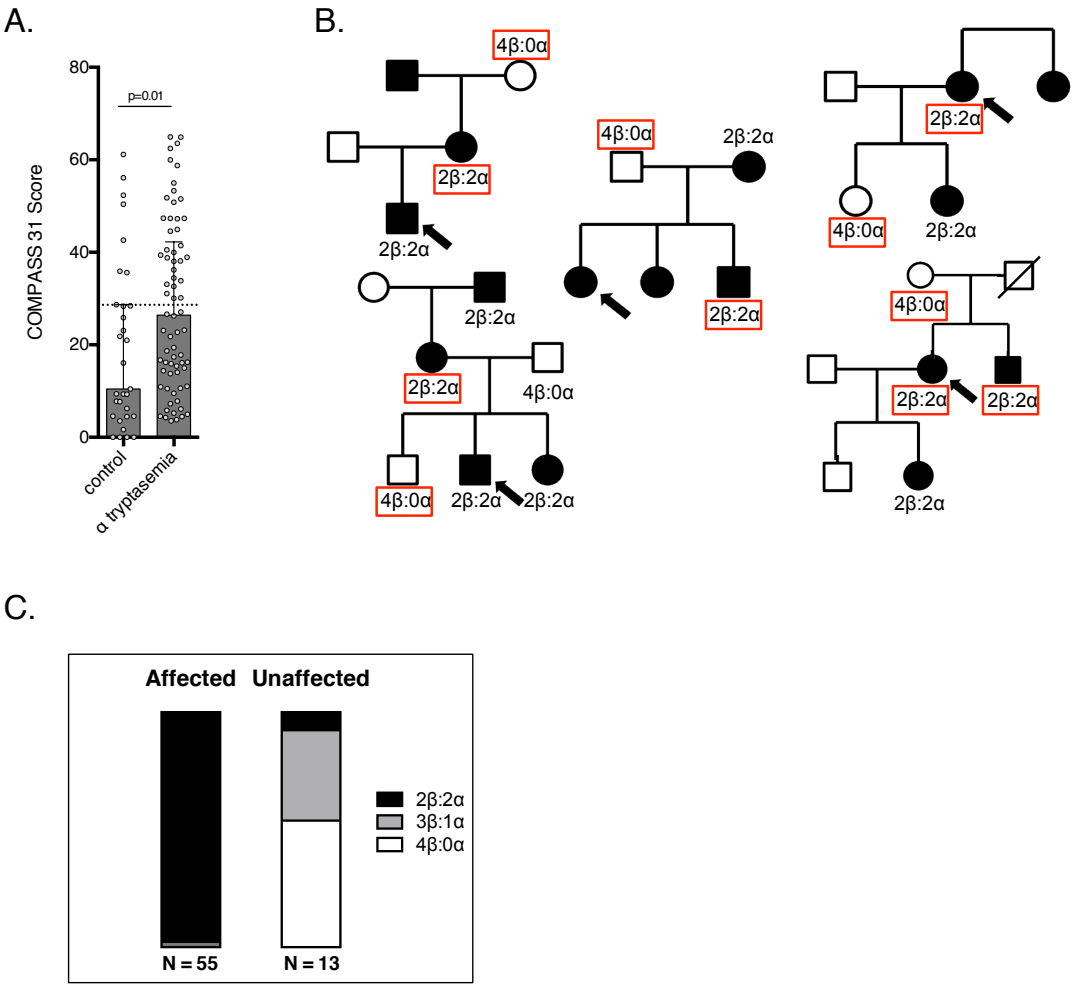


Supplementary Figure 1

Study design for identification of hereditary α-tryptasemia, characterization of associated clinical features, and confirmation of the genetic and clinical features in additional populations.

(**a**) Schematic for evaluation of the referral α-tryptasemia cohort. Families were referred for symptomatic elevation of basal serum tryptase levels without mastocytosis or for familial connective tissue abnormalities in the context of atopy and/or symptoms often associated with mast cell mediators. (**b**) Schematic for evaluation of the NIAID/NIAMS (left) and ClinSeq (right) cohorts. To enrich for individuals with elevated tryptase levels, exome data were reviewed in the 951 individuals enrolled in ClinSeq. Limited coverage of the 16p13.3 locus permitted the selection of 33 individuals with single-nucleotide variants (SNVs) in genes adjacent to *TPSAB1* (*TPSG1* (rs113856625[G>A]) and *CACNA1H* (rs58124832[G>A]) out of 513 with ≥10× coverage at these loci. These SNVs were observed to segregate in 8 of 12 families sequenced with hereditary α-tryptasemia syndrome. Neither SNV has been reported to cause disease; the SNV in *CACNA1H* in combination with another variant has been reported in association with autism spectrum disorders, which were not seen in this cohort (*J. Biol. Chem*. **281**, 22085–22091, 2006). Given that the minor allele frequency (MAF) of these SNVs in Caucasians is approximately 0.06, enrichment was estimated to be between two- and fourfold. An additional 92 patients without these SNVs were selected at random. A cutoff basal serum tryptase level of ≥8 ng/ml was established for further genetic testing based on the range of tryptase levels in the 96 individuals identified with hereditary α-tryptasemia syndrome and the additional 8 individuals identified with hereditary α-tryptasemia in the first and second cohorts, respectively (8–39.5 ng/ml). Of the 25 individuals with basal serum tryptase concentration ≥8 ng/ml, sufficient exome sequence was captured within the *TPSAB1* locus itself to perform bioinformatic genotyping in 16 individuals; the remaining 9 samples had to be excluded. A total of nine individuals were identified with *TPSAB1* duplication of α-tryptase–encoding sequences, seven of whom carried the *CACNA1H* and *TPSG1* variants and two of whom did not. Absence of *TPSAB1* duplication was confirmed by bioinformatic analysis in all 65 individuals with basal serum tryptase concentration <8 ng/ml for whom capture of *TPSAB1* sequence permitted analysis.



Supplementary Figure 2

Increased copy number of α-tryptase–encoding sequences in *TPSAB1* is inherited in families with α-tryptasemia and is associated with higher dysautonomia scores.

(**a**) Distribution of total autonomic dysfunction scores from affected individuals (α-tryptasemia, *n* = 70) and unaffected family members (control, *n* = 31) with inherited *TPSAB1* duplications and triplications, obtained using the standardized COMPASS 31 questionnaire. Data are shown as medians ± interquartile range, Mann–Whitney test. (**b**) Five sample pedigrees showing dominant inheritance of multiple α-tryptase–encoding *TPSAB1* gene copies based on α-tryptase/β-tryptase ratios, which segregate with elevated basal serum tryptase levels (filled symbols). Individuals with normal basal serum tryptase levels are represented with open symbols. (**c**) α-tryptase/β-tryptase ratios obtained from individuals with basal serum tryptase concentration >11.4 ng/ml (affected) and those with normal basal serum tryptase concentrations (unaffected) from 15 families with dominantly inherited elevation of basal serum tryptase levels.

A. Consensus α tryptase

```
α    GTGAGGCCCGGCCAGGCCCACGATGCTCCTCCTTGCTCCCCAGATGCTGAGCCTGCTGCT 840
α    GCTGGCGCTGCCCGTCCTGGCGAGCCGCGCCTACGCGGCCCCTGGTGAGTCCCAGCCGGG 900
α    GTCCACCCTGCCCCTCACCACATTCCACAGGTCAGGGCCTGGGTGGGTTCTGGGGAGGCC 960
α    GGGCTGGCCCCCACACAGGGAAGGGCTGGGCCCAGGCGTGGGGCTGCTTCCTGGTCCTGA 1020
α    CCTGGCACCTGCCCCAGCCCCAGGCCAGGCCCTGCAGCAAGCGGGCATCGTTGGGGGTCA 1080
α    GGAGGCCCCCAGGAGCAAGTGGCCCTGGCAGGTGAGCCTGAGAGTCCGCGGCCCATACTG 1140
α    GATGCACTTCTGCGGGGGGCTCCCTCAGCCACCCCCAGTAGGTGCTGACCGCGGCGCACTG 1200
α    CGTGGGACCGTGAGTCTCCCGGGGGCCTGGAGGGGTGGGCAAGGGCTGGATGTGAGCCCTG 1260
α    GCTCCCGGGTGCTCCTGGGGGCTGCCCAGGGCCCTGAGTGGGATCCTCCGCTGCCCAGGG 1320
```

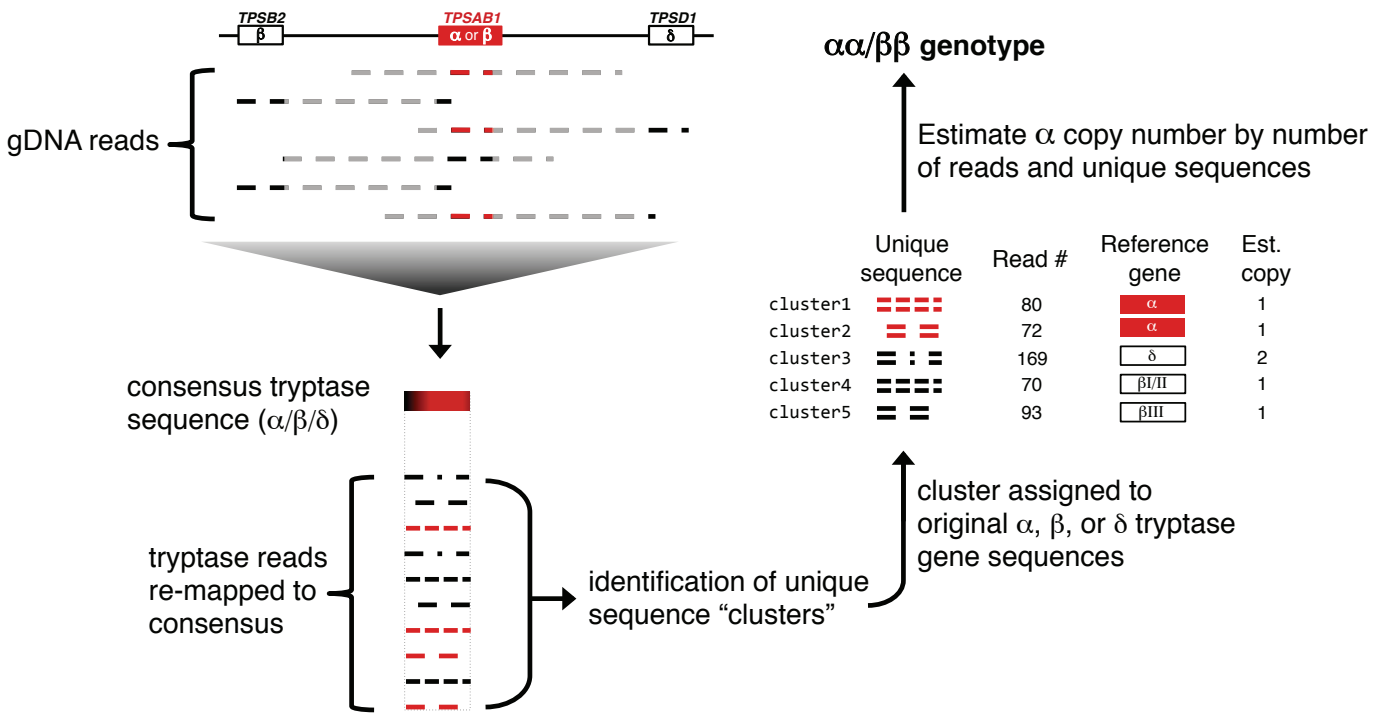
B. Tryptase sequence alignment

```
                                  |START
βIII    GTGAGGCCCGGCCAGGCCCACGATGCTCCTCCTTGCTCCCCAGATGCTGAATCTGCTGCT 840
βI/II   GTGAGGCCCGGCCAGGCCCACGATGCTCCTCCTTGCTCCCCAGATGCTGAATCTGCTGCT 839
α       GTGAGGCCCGGCCAGGCCCACGATGCTCCTCCTTGCTCCCCAGATGCTGAGCCTGCTGCT 840
δ       GTGAGGCCCGGCCAGGCCCACGATGCTCCTCCTTGCTCCCCAGATGCTGAGCCTGCTGCT 840
        ************************************************  ********

βIII    GCTGGCGCTGCCCGTCCTGGCGAGCCGCGCCTACGCGGCCCCTGGTGAGTCCCAGCCGGG 900
βI/II   GCTGGCGCTGCCCGTCCTGGCGAGCCGCGCCTACGCGGCCCCTGGTGAGTCCCAGCCGGG 899
α       GCTGGCGCTGCCCGTCCTGGCGAGCCGCGCCTACGCGGCCCCTGGTGAGTCCCAGCCGGG 900
δ       GCTGGCGCTGCCCGTCCTGGCGAGCCCGGCCTACGTGGCCCCTGGTGAGTCCCAGCCGGG 900
        ************************  ******* ************************

βIII    GTCCACCCTGCCCCTCACCACATTCCACAGGTCAGGGCCTGGGTGGGTTCTGGGGAGGTC 960
βI/II   GTCCACCCTGCCCCTCACCACATTCCACAGGTCAGGGCCTGGGTGGGTTCTGGGGAGGTC 959
α       GTCCACCCTGCCCCTCACCACATTCCACAGGTCAGGGCCTGGGTGGGTTCTGGGGAGGCT 960
δ       GTCCACCCTGCCCCTCACCACATTCCACAGATCAGGGCCTGGGTGGGTTCTGGGGAGGCC 960
        ***************************** *************************

βIII    GGGCTGGCCCCCACACAGGGAAGGGCTGGGCCCAGGCCTGGGGCTGCTTCCTGGTCCTGA 1020 FWD
βI/II   GGGCTGGCCCCCACACAGGGAAGGGCTGGGCCCAGGCCTGGGGCTGCTTCCTGGTCCTGA 1019
α       GGGCTGGCCCCCACACAGGGAAGGGGTGGGCCCAGGCGTGGGGCTGCTTCCTGGTCCTGA 1020
δ       GGGCTGGCCCCCACACAGGGAAGGGCTGGTCCCAGGCGTGGGGCGGCTTCTTGGTCCTGA 1020
        ************************ *** ******* ****** ***** *********

βIII    CCTGGCACCTGCCCCAGCCCCAGGCCAGGCCCTGCAGCGAGTGGGCATCGTTGGGGGTCA 1080
βI/II   CCTGGCACCTGCCCCAGCCCCAGGCCAGGCCCTGCAGCGAGTGGGCATCGTCGGGGGTCA 1079 PROBES
α       CCTGGCACCTGCCCCAGCCCCAGTCCAGGCCCTGCAGCAAGCGGGTATCGTCGGGGGTCA 1080
δ       CCTGGCACCTGCCCCAGCCCCAGGCCAGGCCCTGCAGCAAACGGGCATTGTTGGGGGGCA 1080
        ********************** ************** *  *** ** ** ***** **

βIII    GGAGGCCCCCAGGAGCAAGTGGCCCTGGCAGGTGAGCCTGAGAGTCCGCGACCGATACTG 1140 REV
βI/II   GGAGGCCCCCAGGAGCAAGTGGCCCTGGCAGGTGAGCCTGAGAGTCCACGGCCCATACTG 1139
α       GGAGGCCCCCAGGAGCAAGTGGCCCTGGCAGGTGAGCCTGAGAGTCCGCGACCGATACTG 1140
δ       GGAGGCCCCCAGGAGCAAGTGGCCCTGGCAGGTGAGCCTGAGAGTCCGCGGCCCATACTG 1140
        ********************************************** ** ** ******

βIII    GATGCACTTCTGCGGGGGCTCCCTCATCCACCCCCAGTGGGTGCTGACCGCAGCGCACTG 1200
βI/II   GATGCACTTCTGCGGGGGCTCCCTCATCCACCCCCAGTGGGTGCTGACCGCAGCGCACTG 1199
α       GATGCACTTCTGCGGGGGCTCCCTCATCCACCCCCAGTGGGTGCTGACCGCGGCGCACTG 1200
δ       GATGCACTTCTGCGGGGGCTCCCTCATCCACCCCCAGTGGGTGCTAACCGCGGCGCACTG 1200
        ******************************************** ***** ********

βIII    CGTGGGACCGTGAGTCTCCCGGGGGCCTGGAAGGGTGGGGAAGGGCTGGATGTGAGCCCTG 1260
βI/II   CGTGGGACCGTGAGTCTCCCGGGGGCCTGGAGGGGTGGGGAAGGGCTGGATGTGAGCCCTG 1259
α       CCTGGGACCGTGAGTCTCCCGGGGGCCTGGAGGGGTGGGCAAGGGCTGGATGTGAGCCCTG 1260
δ       CGTGGAACCGTGAGTCTCCTGGGGGCCTGGAGGGGTGGGCAAGGGCTGGATGTGAGCCCTG 1260
        * *** ************* ********** ******* ********************

βIII    GCTCCCGGGTGCTCCTGGGGGCTGCCCAGGGCCCTGAGTGGGATCCTCCGCTGCCCAGGG 1320 BAMHI
βI/II   GCTCCCGGGTGCTCCTGGGGGCTGCCCAGGGCCCTGAGTGGGATCCTCCGCTGCCCAGGG 1319
α       GCTCCCGGGTGCTCCTGGGGGCTGCCCAGGGCCCTGAGTGGGATCCTCCGCTGCCCAGGG 1320
δ       GCTCCCGGGTGCTCCTGGGGGCTGCCCAGGGCCCTGAGTGGGATCCTCCGCTGCCCAGGG 1320
        ************************************************************
```

Supplementary Figure 3

Consensus α -tryptase–encoding sequence and *in silico* alignment.

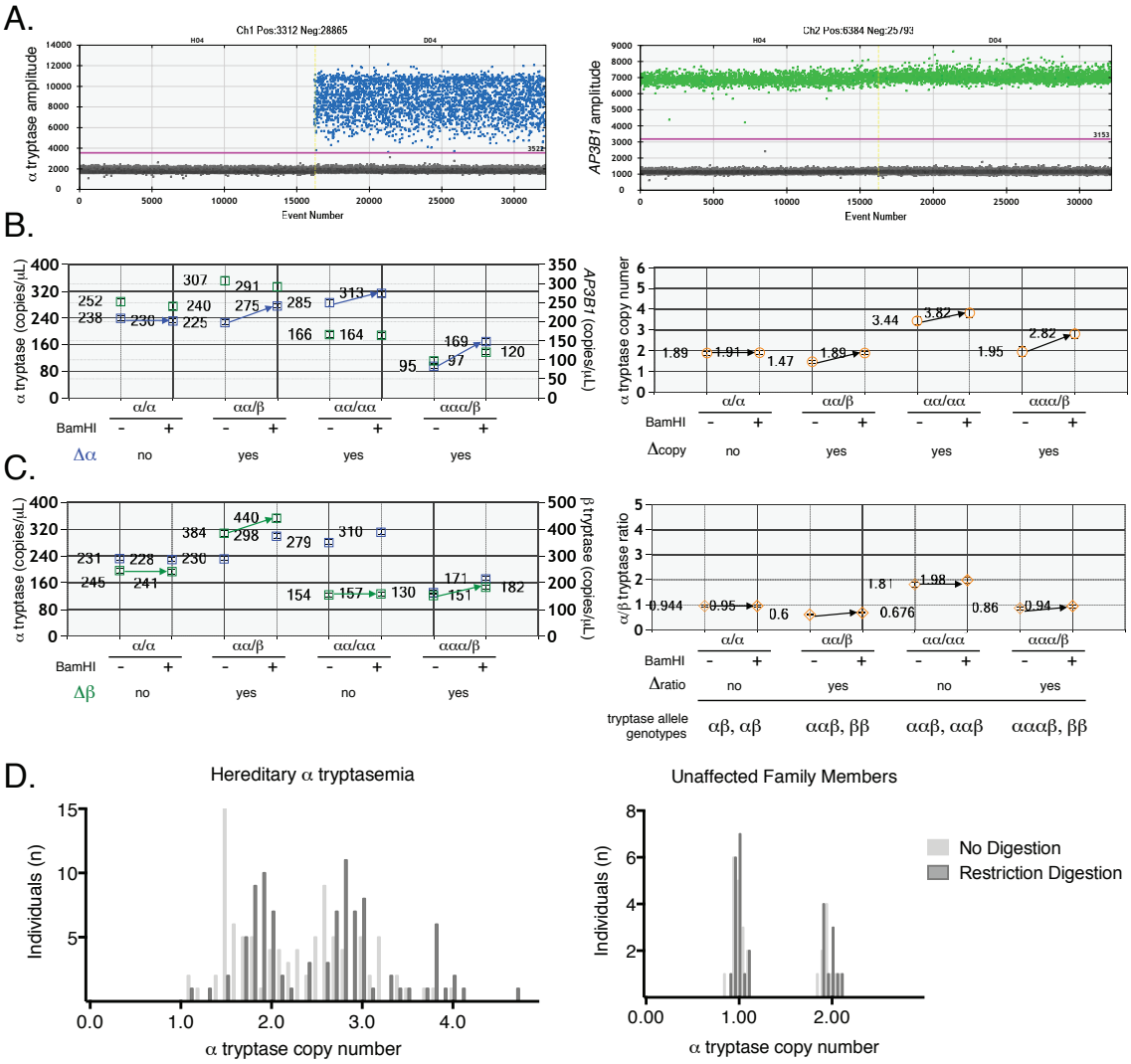
(**a**) Consensus α-tryptase–encoding sequence derived *in silico*. (**b**) Alignment of the sequences encoding α-, βI/II-, βIII-, and δ-tryptase with primer (turquoise), probe (yellow), and restriction site (blue) sequences highlighted.



Supplementary Figure 4

Schematic of the screening bioinformatics algorithm used to estimate copy number of α-tryptase–encoding sequences in *TPSAB1*.

Genomic DNA sequence reads that mapped initially to the general tryptase locus (chr. 16: 1,250,000–1,350,000) were remapped to the α-tryptase-encoding consensus sequence derived *in silico*. Using a computer algorithm, unique sequence 'clusters' with complete internal sequence homology were identified. These clusters were then assigned to one of the unique gene sequences encoding α-, βI/II-, βIII-, or δ-tryptase. On the basis of the number of unique sequences, the number of clusters mapping back to each specific gene, and the number of reads (read #) covering that sequence, an estimated copy number (Est. copy) could be obtained for each gene sequence. Using these estimates, the α-tryptase/β-tryptase genotype encoded at the *TPSAB1* and *TPSB2* loci could be predicted.

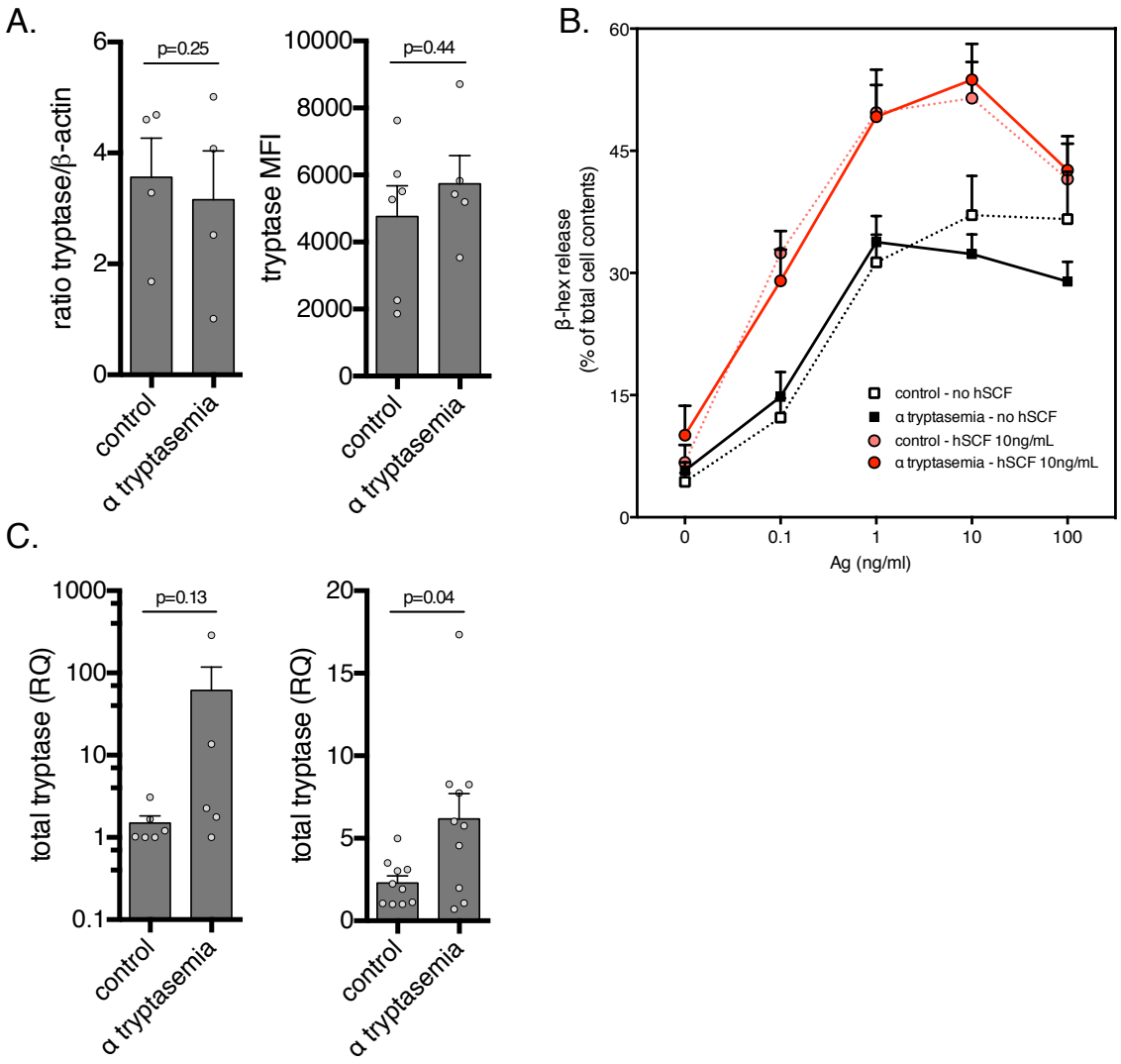


Supplementary Figure 5

Digital droplet PCR assay of α- and β-tryptase.

(**a**) One-dimensional plots of ddPCR data obtained from the genomic DNA of two individuals. Left panel, two individuals, one with an allele encoding α- tryptase at *TPSAB1* and positive (blue) droplets (right) and one with only β-tryptase-encoding alleles at *TPSAB1* (left). Right panel, droplets containing reference gene *AP3B1* for both individuals (green). (**b**) Concentration of α-tryptase–encoding (blue) and *AP3B1* (green) genomic DNA in copies per microliter (left) and corresponding copy number calls for α-tryptase–encoding sequences (right); the genotypes of the four samples are indicated at the bottom. When duplication (αα) or triplication (ααα) of a *TPSAB1* gene encoding α-tryptase is present on a single allele (within 50 kb), a shift (Δα) in the concentration of α-tryptase–encoding sequence relative to the reference is seen with restriction digestion by BamHI, resulting in an increase in calculated copy number (Δcopy), whereas when two different alleles encode α-tryptase, no shift is seen (arrows). (**c**) The ratio of alleles encoding β-tryptase (green) relative to those encoding α-tryptase (blue) (left) also increases (Δβ) following BamHI digestion if two copies of a β-tryptase–encoding sequence are present on a single allele, resulting in a change (arrows) in the α/β ratio (Δratio), thereby allowing for determination of complete tryptase allele genotypes at *TPSAB1* and *TPSB2* (bottom right). (**d**) Histograms of raw copy number calls for α-tryptase–encoding sequences in *TPSAB1* from individuals with hereditary α-tryptasemia (left) and unaffected family members (right), with and without restriction digestion. Individuals with duplications and triplications of α -tryptase–encoding *TPSAB1* sequences in *cis* (on the same allele) initially had an artificially low copy number call owing to droplets containing multiple α-tryptase–encoding sequences that did not independently sort (left). Following brief restriction digestion, a shift toward increased copy number was seen in these individuals, while individuals with two copies of α-tryptase–encoding sequences in *trans* (on separate alleles) demonstrated no

change in copy number as detected by ddPCR (right).



Supplementary Figure 6

Cultured mast cells from individuals with α-tryptasemia express more tryptase transcript, while intracellular protein levels and degranulation activity appear to be normal.

(**a**) Total intracellular tryptase protein expression in mast cells cultured from the peripheral $CD34^+$ cells of individuals with duplication or triplication of α-tryptase–encoding sequences in *TPSAB1* (α-tryptasemia, *n* = 4) and paired cultures (control, *n* = 4) were measured by immunoblot (left; normalized to β-actin) and by flow cytometry following intracellular staining (right) (α-tryptasemia, *n* = 5, control, *n* = 6). Data are combined from five independent culture experiments and are shown as means ± s.e.m., Wilcoxin matched-pairs test. (**b**) Mast cell degranulation in response to increasing antigen (Ag) concentration was measured in cultured mast cells by β-hexosaminidase (β-hex) release in the presence or absence of human stem cell factor (hSCF). Data are from three independent culture experiments (α-tryptasemia, *n* = 4; control, *n* = 5) and are shown as means ± s.d. (**c**) Total *TPSAB1* and *TPSB2* transcripts (total tryptase) were measured in cultured mast cells (five independent paired cultures) and in total PBMCs (*n* = 10 versus 10) from individuals with inherited α-tryptase copy number increases (α-tryptasemia) or paired individuals without extra α-tryptase copies (control) by real-time PCR; data are shown as means ± s.e.m., Wilcoxin matched-pairs test.

Supplementary Appendix

Supplement to: Lyons et al. Elevated basal serum typtase identifies a multisystem disorder associated with increased *TPSAB1* copy number

Table of Contents

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Definitions for reported symptoms and diagnoses

All data presented for ClinSeq® individuals was based upon self-reported symptoms by blinded phone interview; clinical data for the α tryptasemia cohort were collected from both direct patient encounter (n = 55) and serial interviews employing electronic media (n = 41).

Systemic venom reaction: defined by a history consistent with a systemic immediate hypersensitivity reaction following stinging insect envenomation involving the skin diffusely or an additional organ system (skin, lungs, gastrointestinal tract, cardiovascular) (38)

Urticaria/Angioedema: defined by a history and/or clinical observation of urticaria (hives) and/or angioedema (swelling) occurring without a known IgE-mediated trigger (eg. peanut), but could be associated with other allergen-independent triggers (heat, vibration, stress) at least once per month for at least 6 months duration (39)

Flushing/Pruritis: defined as the presence of spontaneous flushing (redness) and/or itching (pruritus) without known specific trigger(s) (as above) occurring at least once per month for at least 6 months duration; these symptoms were frequently accompanied by a sensation of warmth (40)

Chronic gastroesophageal reflux symptoms: defined by a report of chronic symptoms of heartburn and/or regurgitation consistent with reflux of gastric contents and in the absence of dysphagia, odynophagia or known pathology such as EoE (41)

Irritable bowel syndrome [IBS (Rome III)]: all reported IBS was defined by a positive diagnostic score ascertained using the Rome III standardized questionnaire for IBS (42)

Hypermobility: reported only among individuals evaluated in clinic, and over the age of 12 years, defined and quantified using the modified Beighton criteria and a cut-off of ≥ 4 (43)

Scoliosis: defined as radiologic evidence or by direct visual examination in clinic of abnormal lateral spine curvature, or history of physician diagnosis of scoliosis by exam and/or imaging

Retained primary dentition: defined by a history of dental or surgical procedures required to remove retained or impacted (non-molar) primary teeth after 12 years of age

Congenital skeletal abnormality: defined as the presence of one or more of the following: *nail/patella syndrome, severe ankle pronation, severe valgus deformity, neonatal clubbing without cardiopulmonary disease, webbed neck, torticollis, club feet, hip dysplasia, pectus excavatum, high arched palate, syndactyly, genu valgum, severe pes planus, tibial torsion, hyperlordosis,* and *alveolar mandibular hypoplasia*

Pain: because pain severity is difficult to capture and compare, the focus of the pain assessment was on chronicity (44); it was defined as self-reported pain symptoms occurring at least weekly

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for > 6 months in an individuals joints (***arthralgia***), neck or back (***axial***), or localizing to non-musculoskeletal structures (***body pain/headache***) which negatively impacted quality of life (QOL); each of these symptoms is reported independently; quantifying impact on QOL was beyond the scope of this study

Orthostatic symptoms: reported episodic symptoms of racing heartbeat, palpitations, faintness, lightheadedness, presyncope or syncope occurring with changes in posture (eg. sitting to standing) or with acceleration/deceleration (eg. airplane landing, elevator ride) which negatively impacted QOL (45)

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Bioinformatic tryptase haplotype determination

Background

Extracting reads associated with different tryptase genes [*TPSAB1* encoding α (αI) or βI tryptase, *TPSB2* encoding βII or βIII tryptase, and *TPSD1* encoding δ tryptase] and differentiating tryptase isoform sequence is complicated because these different genes have very strong sequence similarity, such that individual read pairs can easily cross-map to multiple reference sequences. α Tryptase-encoding sequence also is very different than the *TPSAB1* sequence present in the reference genome, such that reads that should be assigned to α tryptase frequently map to a combination of all three genetic loci listed above.

The α tryptase sequence used here was extracted from the sequence for a single human sample as follows. All read pairs that mapped to the general tryptase region (Chr16:1,250,000-1,350,000), but that had a significant number of mismatches (>=3) were selected. These reads were then assembled using Velvet to obtain a novel sequence that was then iteratively refined to produce a unique sequence (46). It was subsequently confirmed that this sequence was the α tryptase-encoding sequence, which is only 97% identical to the reference *TPSAB1* and *TPSB2* sequences. By contrast, the reference sequences for *TPSAB1* and *TPSB2* are 98.8% identical to each other (Fig. S3A, B).

With standard short-read mapping methods, it is very difficult to assess the relative amount of each of the different isoforms and to determine their genotypes. Too many reads will map equally well to two or more of the isoforms. This has the effect of generating false positive variant calls. To work around this problem, we identified a short segment of genomic sequence that was highly but not perfectly conserved, across the four genes. We generated a consensus sequence from this alignment so that reads mapping perfectly or with a few mismatches to any

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one of the individual genes would still map well to the consensus sequence. Even with this technique, βI and βII isoforms could not be resolved from one another within the consensus region.

To dissect the genotype and amount of each of the tryptase genes for a given sequence sample, we extracted all reads that originally mapped to the tryptase region (Chr16:1,250,000-1,350,000), and re-mapped them to the short consensus region. This resulted in an alignment of the reads for each of the four genes onto a single common reference. Then a custom method was devised to classify the read pairs into separate clusters, or haplotypes. The clustering technique devised required perfect compatibility amongst all members of the cluster. Read pairs with even a single sequencing error were excluded with this method. The clustering process started with those pairs of sequences that have the most informative overlap. Doing this reduced the chances of merging reads that did not come from the same haplotype.

Once the sequence clusters were determined, they were mapped back to the four original sequences, assigned to one matching it best, and variants relative to that best-match sequence were determined. An estimate of copy number was derived from the number of reads in the cluster (Fig. S4). One individual was estimated to have 6 α tryptase gene copies based upon coverage, but with only 2 unique sequences. All other individuals with confirmed increased copy number were estimated to have 4 or fewer α tryptase-encoding copies, with at most 3 unique α tryptase sequences. These findings were confirmed by manual exploration of the sequence data.

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Procedure

Details are given in the scripts. Briefly, the procedure is as follows:

1. Extract 'tryptase' reads from the BAM file. It is also possible to simply map all reads to the consensus sequence, but it should not be necessary, and it is much more efficient to narrow in on the tryptase region immediately.
2. Convert reads back to FASTQ, and collate as read pairs.
3. Map to the consensus sequence using BWA.
4. Cluster mapped reads using a custom python script written for that purpose. This will generate a collection of generally full-length (540bp) sequences, and well as a coverage estimate for each.
5. Map the resulting sequences to the separate tryptase gene sequences to identify which sequence corresponds to which gene, as well as what variants were found.
6. Manually assess the results considering the overall coverage of the experiment to assign the most likely allele count for each. In some cases, the clustering algorithm will be confused and generate more haplotypes than there truly are. These cases can usually be figured out by manual inspection of the read clusters.

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Script to ascertain tryptase content from sequence data

```
Software versions: samtools 1.2, bwa 0.7.12
Required reference sequences: CONS.fa, TPS.fa, indexed with BWA
Alternative, longer reference sequences: CONS1.fa, TPS1.fa

#########################

# SM= sample identifier
SM=1940NIH

# Extract reads mapping to the general tryptase locus from original BAM file
samtools view -b -F0xF0C $SM.bam 16:1250000-1350000 > $SM.tryptase.bam

# Convert to interleaved FASTQ
samtools bamshuf -Ou $SM.tryptase.bam temp |samtools bam2fq - > $SM.tryptase.fq

# Map reads to consensus sequence, retaininly only mapped reads
bwa mem -pM -R '@RG\tID:1\tSM:'$SM CONS.fa $SM.tryptase.fq |samtools view -F0xF0C -S -h - >
$SM.cons.sam

# Cluster mapped reads into distinct haplotypes
cat $SM.cons.sam \
  |awk '$10!~/N/' \
  |python parseHaplotypes.py \
  > $SM.fa

# Resulting FASTA file has sequences for each cluster found. The FASTA header for each sequence is
formatted as:

>cluster#_count_depth {string with int(coverage/10) for each position of the sequence}
```

The coverage distribution can give a quick clue as to whether something went wrong with the clustering. Ideally, it should be uniform across the sequence.

```
# Align to reference to view contigs aligned
cat $SM.fa \
  |sed 's/ .*//' \
  |sed 's/^X*//' \
  |sed 's/X*$//' \
  |tr X N \
  |bwa mem -M TPS.fa - \
  |awk '$1!~/_[0-9]_/' \
  > $SM.consx.sam

cat $SM.cons.sam |grep -v ^@ \
  |cut -f1,3,4,6,12-13|tr _ "\t" \
  |awk '$3>5' \
  |sort -k4,4 -k3nr \
  > $SM.output.txt

#########################################################
$SM.output.txt is the main output file.
```

Sample output data file

cluster1	208	95	βI/II	1	540M	NM:i:2	MD:Z:178T112C248
cluster4	109	52	α	1	540M	NM:i:5	MD:Z:86G0C117G166C67G99
cluster5	89	37	α	1	540M	NM:i:4	MD:Z:86G0C7C344G99
cluster2	118	57	δ	1	540M	NM:i:1	MD:Z:382C157
cluster3	110	47	δ	1	540M	NM:i:5	MD:Z:86C0G7T315G9G118

This sample has two different alleles of *TPSD1* (δ), two different alleles of α tryptase, and two of the same allele (at least over this 540bp) for βI/II. In each case, there are a few (1-5) SNVs relative to the reference sequences. Interestingly, this sample doesn't have any βIII according to this analysis. This is consistent with reports that βIII is restricted to the *TPSB2* locus and limited to an allele containing βI at *TPSAB1* (47); here the α tryptase copies were on separate alleles containing β (inferred as βI in this case) detected in an unaffected individual without copy number increases of α tryptase.

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Digital droplet PCR assay for allele-specific α/β tryptase genotyping

In order to perform directly quantify the copy number of α and β tryptase-encoding alleles, the aligned consensus sequences were interrogated and a region with single nucleotide variation between α, β, and δ-encoding tryptase sequences was targeted for α and β tryptase-sequence specific probe design (Fig. S3B, highlighted in yellow). Primers conserved across all tryptase species were also designed (Fig. S3B, highlighted in turquoise), and we confirmed that the α tryptase probe only hybridized with α tryptase sequence (Fig. S5A).

To test whether α tryptase-encoding *TPSAB1* duplications were occurring within the tryptase locus, we used intrinsic properties of our reagents and digital droplet PCR (ddPCR). The technology partitions genomic DNA (gDNA) into small droplets with some but not all droplets containing the target of interest. This droplet count is then compared to a reference gene, and copy number is determined with the assumption that the target will follow a Poisson distribution. Thus, for accurate copy number determination of any target, all copies must independently distribute into droplets. The tryptase locus is estimated to be only 36kb, and the maximum size gDNA fragment obtained by the extraction kit (Qiagen DNeasy Cat No./ID 69506) is 50kb, with average size ~30kb. Thus if the duplication was occurring outside of the locus (eg. further than 50kb) no effect on positive droplet count by restriction digestion with BamHI should be seen (Fig. S3B, highlighted in blue). However, because an increase in droplet count and subsequent copy call was observed, it could be deduced that additional α tryptase-encoding copies were occurring at the locus (Fig. S5B). This same method and logic was applied to copy number detection for β tryptase-encoding sequence, and by combining the 2 data sources, and by calculating the ratio of α/β tryptase sequence copy number, complete α/β allele genotypes for *TPSAB1* and *TPSB2* could be generated for most individuals (Fig. S5C); some

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more complex allele combinations could only be completely resolved by inheritance patterns of deduced genotypes.

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TABLES

Supplementary Table 1. Demographics of individuals from 35 families identified with Hereditary α tryptasemia syndrome.

	TOTAL (n = 96)
Age, mean (range)	39 years (2-89)
Gender, n (%)	
Male	36 (37.5)
Female	60 (62.5)
Ethnicity, n (%)	
Caucasian	96 (100)

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Supplementary Table 2. Demographics of individuals with α tryptase-encoding *TPSAB1* duplications (αα) and those without duplications (WT) within the ClinSeq® cohort.

	TOTAL (n = 91)	αα (n = 9)	WT (n = 82)
Age, mean (SD)	61.7 years (±5.7)	60.1 years (±6.6)	61.9 years (±5.7)
Gender, n (%)			
Male	42(46)	4 (44)	38 (46)
Female	49 (54)	5 (56)	44 (54)
Ethnicity, n (%)*			
Caucasian	85 (93)	9 (100)	76 (93)
African-American	3 (3)	-	3 (4)
Other	3 (3)	-	3 (4)
Hispanic/Latino	8 (9)	-	8 (10)
Serum tryptase, median (range)	-	14.2 ng/mL (8.2-30)	4.4 ng/mL (1.3-9.8)

*Some individuals identified as being more than one ethnicity.

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Supplementary Table 3. Self-reported individual clinical features of ClinSeq® participants identified with α tryptase-encoding *TPSAB1* duplications on a single allele.

	Subject								
Manifestation	1	2	3	4	5	6	7	8	9
Systemic venom reaction*	■		■						
Flushing/Pruritis	■	■	■	■	■				
IBS (Rome III)	■	■			■				
Chronic gastroesophageal reflux symptoms	■	■		■	■	■		■	■
Congenital skeletal abnormality†						■			
Scoliosis	■	■		■					
Retained primary dentition	■		■			■			
Hypermobility (Beighton score ≥4)‡	ND	■		ND	ND	ND	ND	ND	ND
COMPASS 31§	■	■	■	■					
Joint Pain		■	■		■		■		
Body Pain/Headache	■	■	■						
Sleep disruption	■	■							

Filled square (black) indicates presence of reported symptoms. *Systemic immediate hypersensitivity reaction consistent with IgE-mediated to stinging insect, as described in the Supplemental Note; †Spina bifida occulta, congenital absence of spinous process, pectus excavatum, and tibial torsion; ‡Only two adult individuals were available for clinical evaluation following blinded-interview results; §Individuals with a composite score above the upper 95% CI of median established in a healthy control cohort without increased α tryptase copy number; ND – not determined.

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Supplementary Table 4. Primer and probe sequences for α and β tryptase genotyping.

Oligonucleotide target	Sequence
TPSAB1/*TPSB2* - FWD	5'-TCCTGACCTGGCACCTGC-3'
TPSAB1/*TPSB2* - REV	5'-GACTCTCAGGCTCACCTGCCA-3'
α probe	5'-CTGCAGCAAGCGGGTATCGTC-3'
β probe	5'-CTGCAGCGAGTGGGCATCGT-3'

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